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Abstract: The objective of this study was to investigate the gender-specific differences in peak torque (PT), muscle stiffness (MS) and musculoarticular stiffness (MAS) of the knee joints in a young active population. Twenty-two male and twenty-two female recreational athletes participated. Peak torque of the knee joint extensor musculature was assessed on an isokinetic dynamometer, MS of the vastus lateralis (VL) muscle was measured in both relaxed and contracted conditions, and knee joint MAS was quantified using the free oscillation technique. Significant gender differences were observed for all dependent variables. Females demonstrated less normalized peak torque (mean difference (MD) = 0.4 Nm/kg, $p = 0.005$, $\eta^2 = 0.17$), relaxed MS (MD = 94.2 N/m, $p < .001$, $\eta^2 = 0.53$), contracted MS (MD = 162.7 N/m, $p < .001$, $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, $p < .001$, $\eta^2 = 0.23$) than males. MAS increased linearly with the external load in both genders with males demonstrating a significantly higher slope ($p = 0.019$) than females. The observed differences outlined above may contribute to the higher knee joint injury incidence and prevalence in females when compared to males.

***Title Page with full affiliation details (including address)**

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Abstract:

The objective of this study was to investigate the gender-specific differences in peak torque (PT), muscle stiffness (MS) and musculoarticular stiffness (MAS) of the knee joints in a young active population. Twenty-two male and twenty-two female recreational athletes participated. Peak torque of the knee joint extensor musculature was assessed on an isokinetic dynamometer, MS of the vastus lateralis (VL) muscle was measured in both relaxed and contracted conditions, and knee joint MAS was quantified using the free oscillation technique. Significant gender differences were observed for all dependent variables. Females demonstrated less normalized peak torque (mean difference (MD) = 0.4 Nm/kg, $p = 0.005$, $\eta^2 = 0.17$), relaxed MS (MD = 94.2 N/m, $p < .001$, $\eta^2 = 0.53$), contracted MS (MD = 162.7 N/m, $p < .001$, $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, $p < .001$, $\eta^2 = 0.23$) than males. MAS increased linearly with the external load in both genders with males demonstrating a significantly higher slope ($p = 0.019$) than females. ~~It is hypothesized that t~~The observed differences outlined above may contribute to the higher knee joint injury incidence and prevalence in females when compared to males.

Introduction

Epidemiological research has reported that female athletes have an increased risk of lower limb musculoskeletal sports related injuries when compared to their male counterparts (Jones et al., 1993, Messina et al., 1999). This observation is particularly relevant in relation to anterior cruciate ligament (ACL) injuries and patellofemoral pain (PFP). Female soccer players have been reported to have a 2-3 times higher risk of ACL injuries when compared to males (Walden et al., 2011)~~.~~ ; ~~T~~this is also seen ~~in female athletes~~ in other high velocity, intermittent sports such as basketball and volleyball (Hewett, 2000). PFP is a prevalent lower limb musculoskeletal disorder, observed in young, physically active female athletes (Heintjes et al., 2003, Natri et al., 1998), and is associated with reduced participation in field and court based sports. Furthermore, it may precipitate the onset of patellofemoral osteoarthritis (Utting et al., 2005), as well as being potentially linked to non-contact ACL injury risk (Myer et al., 2014).

Factors that are thought to contribute to gender differences in the incidence and prevalence of knee joint injuries include; differences in the mechanical properties of the knee joint ligaments, knee joint kinematics during landing, cutting and pivoting, as well as skeletal alignment (Bonci, 1999, Harner et al., 1994, Rosene and Fogarty, 1999). During sport related activities, joint loads increase and knee joint stability is dependent upon activation of the dynamic muscular constraint system, aimed at protecting joints against injury. Kim et al. (Kim et al., 2011) summarized from previous studies that co-contraction of agonist and antagonist muscles is important for joint stabilization during dynamic movement; the amount of co-contraction could significantly influence the resultant torque at the knee joint . Billot et al. indicated that agonist-antagonist muscles have a common descending drive control (Billot et al., 2014). Imbalance of quadriceps and hamstring strength (hamstring/quadriceps ratio < 0.6) has been reported as a contributing factor to non-contact knee injuries (Kim et al., 2011). Furthermore, neuromuscular imbalance of decreased hamstring activation relative to quadriceps activation is also well documented as a risk factor for ACL injury (Alentorn-Geli et al., 2009). The role of hamstring muscles during landing or cutting is to provide a counterbalancing force to resist the relatively higher quadriceps force; h~~H~~igher quadriceps muscle activity and altered co-activation patterns in females have been inferred to change the knee joint loads and thereby increase their risk for knee injury (Krishnan et al., 2009). In this context, strength is only one component of injury mechanism; neuromuscular function is actually the primary contributor to the higher risk of non-contact lower limbs injuries in females when compared to males. In contrast, stiffness is a more comprehensive variable which represents the shock absorption characteristics of an individual muscle-tendon unit, joint, or system (Watsford et al., 2010). ~~Indeed, muscle stiffness is a primary control variable related to k~~~~Knee joint stability is mainly determined by muscle stiffness (Needle et al., 2014). Additionally, stiffness is a primary determinant of the shock absorption characteristics of an individual muscle-tendon unit, joint, or system (Watsford et al., 2010).~~ A recent consensus paper ~~published by Shultz and colleagues~~

(Shultz et al., 2012) advocated that further insight into the dynamic-restraint systems of the knee joint beyond absolute strength is ~~are~~ required to understand more comprehensively the potential mechanisms associated with the observed gender disparity in knee joint injuries amongst athletes, with the authors recommending that further research regarding knee joint stiffness is warranted.

Musculoarticular stiffness (MAS), assessed with the free-oscillation technique, is a comprehensive measurement incorporating the stiffness of the muscle-tendon unit, surrounding articular surfaces, ligaments, and skin. The same technique can be applied to a single muscle using a specific device, thus obtaining a more localized measurement of muscle stiffness (MS) than MAS evaluation ~~in joint~~. It has been advocated that some level of stiffness is beneficial to enhance athletic performance, however too much or too little stiffness may increase the risk of injury (Butler et al, 2003). Further, whilst an elevated level of stiffness appears to be beneficial for rapid stretch-shortening cycle (SSC) movements, during relatively slow SSC movements a more compliant structure can better utilize the eccentric pre-stretch and cushion the impact (Pruyn et al, 2014). That's why MS and MAS have the potential to play crucial roles in neuromuscular control of joint stability, injury prevention and athletic performance (Ditroilo et al., 2012, Ditroilo et al., 2011b). ~~The level of stiffness contributes to the ability to attenuate excessive external forces, which is why MS and MAS have the potential to play crucial roles in neuromuscular control of joint~~

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~~stability, injury prevention and athletic performance (Ditroilo et al., 2012, Ditroilo et al., 2011b).~~

To the present authors knowledge, no studies to date have concomitantly measured and compared knee joint MAS and quadriceps MS in male and female recreational athletes. ~~In the present study, v~~Vastus lateralis (VL) was utilized as representative of the quadriceps muscle ~~in accordance with previous research by Cafarelli~~ (Cafarelli, 1977). Thus, the aim of the present study was to concurrently investigate MAS of knee joints and MS of VL in young male and female athletes. It was hypothesized that females would be characterized by lower knee joint MAS and MS of the VL when compared to males, which could help to explain an important mechanism linked to gender disparities in knee joint musculoskeletal injuries.

Methods

Participants

Twenty-two male (age = 26.7 ± 2.6 years, ~~height~~ stature =1.77~~.2~~ ± 0.06~~.6~~7 ~~c~~m, body cmass = 72.6 ± 9.1 kg, BMI = 23.1 ± 2.4 kg/m^2) and twenty-two female recreational athletes (age = 23.8 ± 4.1 years, stature ~~height~~ = 1.65~~4.8~~ ± 0~~7~~.08~~7 c~~m, body mass = 63.0 ± 12.0 kg, BMI = 23.1 ± 3.5 kg/m^2) volunteered to participate. The study protocol was approved by the University Human Research Ethics Committee, and all participants signed consent forms. The specific inclusion criteria were: (1) recreational athletes who participated in organized sports ; (2) aged: 18-35 years; (3)

BMI ≤ 25 (if a participant's BMI was > 25, body fat ≤ 25% (males) or 35% (females) (assessed via skinfold thickness) were deemed acceptable (Ho-Pham et al., 2011)); (4) no recent significant soft-tissue injury to the lower limbs in the last 6 months; (5) no reported medical condition that could influence performance. Furthermore, participants were also screened using a medical history questionnaire (Ditroilo et al., 2011a) and the Physical Activity Readiness Questionnaire form.

Study design

Each participant was required to visit the laboratory on one occasion and undergo the following evaluations: (1) peak torque (PT) testing of their right knee joint extensor musculature; (2) relaxed MS testing of their right VL; (3) contracted MS testing of their right VL; (4) contracted MAS testing of their right knee joint.

Peak Torque (PT)

Each participant underwent PT testing of their right knee joint extensor musculature on a dynamometer (Bodymax Fitness, Clydebank, UK). The participant was seated on the dynamometer with their; hip flexed at 105°and their right knee flexed at 80° (where full extension represents 0°) (Ditroilo et al., 2012), with the lateral femoral condyle aligned with the axis of the dynamometer. The force transmission point was a bar that was positioned anteriorly to the participant's lateral malleolus. The machine was equipped with a load cell (Leane International, Parma, Italy, measurement range: 0-500 kg, output: 2.00 mV/V) applied in series with the plane of force application.

The load cell was secured to the leg-extension machine with a chain. This prevented movements of the bar and therefore allowed an isometric contraction when the participant attempted to extend their leg. Participants were stabilized with straps at the pelvis to avoid movements towards hip extension during the test. Furthermore, to minimize any contribution from the upper body, participants were required to cross their hands across their body throughout. After familiarization with the procedures, participants were instructed to produce a maximum voluntary isometric contraction (MVIC) of their knee joint extensor musculature, as quickly as possible for approximately 3 seconds. Each participant was required to perform three MVICs, with the highest value recorded being used to determine the load with which MAS was assessed. During performance of each MVIC, strong verbal encouragement and visual target stimulation were provided to motivate maximal contraction. The force signal was sampled at 1000 Hz and stored on a PC using a 16 bit A/D converter data acquisition system (Biopac Systems, Inc. Goleta, CA, USA). Prior to data analysis, the signal was filtered using a 5-ms moving average. The force signal was then multiplied by the individual lever arm length to convert it into torque (Nm). The highest torque value was identified as PT, which was normalized to body mass of each individual (Pincivero et al., 2003) for further analysis.

Muscle stiffness (MS)

MS of the VL muscle was measured using a device incorporating a probe and an accelerometer (Myometer, Myoton-3, Müomeetria AS, Tallinn, Estonia) sampled at

3200 Hz. During MS recordings, the subjects were seated in the same position used for MVIC measurements. The probe was manually positioned perpendicular to the muscle belly with the recording site being 2/3 the distance along a line measured from the anterior superior iliac spine to the midpoint on the lateral side of the patella. The probe was gently lowered onto the muscle belly of the VL with a resultant automatic mechanical impact being delivered to the muscle (duration of 15 ms, a force of 0.3-0.4 N and a local deformation in the order of a few millimeters) (Ditroilo et al., 2012). The damped natural oscillations were recorded by the accelerometer within the probe giving an instantaneous digital output of the MS. Five consecutive measurements were taken during relaxed (no external load) and contacted (external load = 30% MVIC) (Fig. 1.) conditions. The average of the five measurements was used for later analysis.

Musculo-articular stiffness (MAS)

MAS of each participant's right knee joint was measured using a technique previously published by Ditroilo et al., 2012 (Fig. 2.). Participants sat in the same position used previously for MVIC assessments. To quantify submaximal MAS stiffness, the participants were required to support a load corresponding to 30% of MVIC on the anterior distal portion of their lower leg. An external perturbation of 100-150N was applied to the bar by the investigator and the ensuing oscillations were recorded by a uniaxial accelerometer (Crossbow, Milpitsa, CA, USA) attached to the distal end of the lever arm of the leg-extension dynamometer. Accelerometer data were sampled at

1000 Hz and recorded on a personal computer using a 16-bit A/D converter. A Butterworth low-pass filter (third order) with a cutoff frequency of 4 Hz was used to filter the signal. Each participant completed five MAS trials separated by a 1-min rest period, with the average of the three trials being used for analysis. Considering the positive relationship between the active joint stiffness and the applied load, stiffness gradient, defined as the ratio of the two parameters, was subsequently calculated afterwards and utilized as an independent variable in the statistical analysis (Gardner-Morse et al., 1995).

Statistical Analysis

Independent samples *t*-tests (two tailed) were undertaken to investigate differences between males and females on the following four dependent variables: (1) PT~~peak torque~~; (2) relaxed MS; (3) contracted MS; (4) MAS. Statistical analyses were conducted in IBM SPSS Statistics 20 (IBM Ireland Ltd, Dublin, Ireland). To account for the number of analyses undertaken, statistical significance was set a priori at $p \leq 0.0125$ (Bonferroni adjustment). Furthermore, a one-way between-groups analysis of covariance (ANCOVA) was conducted to investigate differences in stiffness gradient across genders with the external load as the covariate; the level of significance was set at $p < 0.05$.

Results

A significant difference was observed between males and females in; normalized PT~~peak torque~~ (PT~~peak torque~~/ body mass) (males 2.8 ± 0.4 Nm/kg, females 2.4 ± 0.4 Nm/kg (Fig. 3.); $t(42) = 2.96$, $p = 0.005$), relaxed MS (males 364.4 ± 52.0 N/m, females 270.3 ± 33.3 N/m (Fig. 4.); $t(42) = 6.90$, $p < .001$), contracted MS (males 495.1 ± 71.0 N/m, females 332.3 ± 85.4 N/m (Fig. 5.); $t(42) = 6.9$, $p < .001$) and MAS (males 1450.1 ± 508.0 N/m, females 1028.0 ± 227.3 N/m (Fig. 6.); $t(42) = 3.55$, $p < .001$).

The magnitude of the difference in means was also large for; normalized ~~peak torque~~ PT (mean difference (MD) = 2.3 Nm/kg, 95% CI: 0.1 to 0.6, $\eta^2 = 0.17$), relaxed MS (MD = 94.2 N/m, 95% CI: 66.6 to 121.7 $\eta^2 = 0.53$), contracted MS (MD= 162.7 N/m, 95% CI: 114.9 to 210.5, $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, 95% CI: 179.5 to 664.8 $\eta^2 = 0.23$)

~~The one-way ANCOVA P~~preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances and regression slopes, and reliable measurement of the covariate before one-way ANCOVA was ~~.~~processed. After adjusting for external load, there was significant difference for MAS between the two groups, $F(1, 42) = 6.02$, $p = 0.019$, with males having a steeper stiffness gradient slope than females (Males, Y= 36.92X-786.51, $r^2 = 0.80$; Females, Y= 18.32X+224.49, $r^2 = 0.33$). (Fig. 7.).

Discussion

~~This investigation aimed to identify whether differences in the stiffness characteristics of the knee joint exist between young recreationally athletic males and females. To the best of the authors' knowledge, this is the first study to concurrently measure MS of the VL and MAS of the knee joint (extensor) in young recreational athletes.~~ The primary findings were that females have lower relaxed and contracted MS of the VL and were characterized by lower knee joint MAS, which are important mechanisms underlying gender disparity~~. It is possible that these observed stiffness discrepancies across genders may contribute to higher rates of knee injury incidence and prevalence observed in female athletes~~.

MS ~~is a localized evaluation of the muscle's ability to resist external load. It~~ is influenced by geometry (physiological cross-sectional area, PCSA) (Foure et al., 2012) and hence muscle mass (muscle mass= PCSA*fiber length* ρ) (Narici et al., 1992), as well as intrinsic properties (actin-myosin cross-bridge, and protein titin) (Proske and Morgan, 1999~~, Wu et al., 2000~~). Therefore, gender differences in relaxed MS could be attributable to the fact that males have a larger PCSA, greater~~more~~ muscle mass and thereby~~thus a greater amount~~more ~~of~~ muscle fiber cross-bridges (Blackburn et al., 2004) and titin than females. Gajdosik et al. (Gajdosik et al., 1990) for instance suggested that higher hamstring stiffness values in males were ascribed to greater muscle mass compared to their female ~~, whilst Blackburn~~counterparts. Blackburn et al.~~, 2004,~~ also postulated that greater thigh segment mass in males could

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be responsible for observed gender differences in passive knee flexor stiffness,~~.~~ f~~F~~urthermore, increased muscle mass in males implies more passive connective tissue, and hence a greater number of collagen fibers for lengthening resistance when compared to ~~those in~~ females, leading to increased passive stiffness (Blackburn et al., 2004). In addition, in contracted muscles, the amount of cross-bridges formed should also be considered, as contracted MS has been found to be proportional to contractile forces in muscle (Needle et al., 2014). Previous study~~ies~~ has~~ve~~ shown that males are stronger than females (~~Hannah et al., 2012,~~ Wojtys et al., 2002a), a finding also confirmed by the present study, whereby males produced significantly higher normalized PT~~peak torques~~ values compared to females (2.8 ± 0.4 Nm/kg vs 2.4 ± 0.4 Nm/kg).

Males were also found to have greater MAS compared to females, which is consistent with conclusions of a previous study (Blackburn et al., 2009). Sinkjaer et al. (Sinkjaer et al., 1988) divided MAS into two parts: the intrinsic component (deformation and breakdown of actin-myosin filament cross-bridges) and the reflexive component (occurs after the establishment of intrinsic portions during rapid muscle stretches). The intrinsic component increases linearly with background torque (pre-activation) (Mrachacz-Kersting and Sinkjaer, 2003) which is the external stretch on quadriceps; whilst the reflexive component is integrated by the central nervous system~~ (CNS)~~, and accounts for approximately 50% of the total stiffness (Hinsey, 2011). Muscle contraction plays an essential role in joint stiffness (Needle et al., 2014), leading to a

2-4 times increase in knee joint stability (Markolf et al., 1976). Furthermore, studies have reported that active joint stiffness is proportional to the force generated by muscles (Morgan, 1977~~, Morgan et al., 1978~~). Thus, factors related to muscle force production, such as geometric mechanisms (Granata et al., 2002b), cross-bridge mechanics and material qualities (Hinsey, 2011) are promising explanations for the gender differences in joint stiffness found in the current investigation.

In addition to the aforementioned mechanisms, knee joint stiffness properties can also be influenced by hormones, specifically free testosterone (FT) (Bell et al., 2012, Granataet al., 2002b). An early study showed that when compared to females, male adults possess approximately 7-8 times more FT (Southren et al., 1965). It has been observed that an inverse relationship exists between FT and time to 50% peak torque; with shorter time to 50% ~~peak torque~~PT being more advantageous to overall joint stability (Bell et al., 2012, Blackburn et al., 2009). Bell et al., 2012, have reported that a negative relationship exists between estrogen and MAS, offering some explanation for the lower MAS observed in females. We hypothesize that this is the case for the present study although no experimental measurements were carried out.

Stiffness gradient is an essential tool to describe active stiffness characteristics. The results of the current study demonstrated a significantly higher stiffness gradient in males in comparison to females, indicating that when an applied moment increases, joint stiffness subsequently increases, and males manifest a higher degree of increased stiffness. Therefore, it is reasonable to assume that males are characterized by greater ability to resist external loads which has implications for injury risk in females. The observed difference in stiffness gradient between males and females is also supported by the findings of Granata et al., 2002b which reported that stiffness increased with the external load, and there was a significant difference in slope of linear regressions between stiffness and applied load with females demonstrating a reduced regression slope .

Joint stiffness parameters are integrated by the CNS internally and exhibit mechanical characteristics externally. As a consequence, it is an important variable capable of comprehensively representing joint stability and muscle performance. A higher degree of stiffness may provide more resistance to external load during functional performance and hence protect joints from musculoskeletal injury (Granata et al., 2002a). A decrease in joint stiffness or MS reduces structures' capacity to resist external applied loads, and hence the gender differences in stiffness observed in the present study could help explain the higher risk of lower-limb injuries in females. It could also point out one possible solution for preventing injuries in females and males. Training; such as weight (Kubo et al., 2007)~~, isometric (Burgess et al., 2007), eccentric (Pousson et al., 1990),~~ and plyometric training (Spurrs et al., 2003) have all been suggested to be beneficial for stiffness augmentation. In the future, it is important to investigate what kind of training is best for stiffness enhancement.

Limitations of this study include; not measuring the participants' testosterone and estrogen levels, and also not controlling females' menstrual cycle due to time and financial limits. The effect of menstrual cycle hormone fluctuations on stiffness properties and the injury occurrence is still controversial. The study of Eiling et al. (Eiling et al., 2007) indicated significant effect of estrogen levels on musculotendinous stiffness at the time of ovulation when compared to the menstrual and follicular phase; and more acute ACL tears were reported in females during mid-cycle by Wojtys et al. (Wojtys et al., 2002b). However, Bryant et al. (Bryant et al., 2011) attested no significant leg stiffness difference between non-MOCP (monophasic oral contraceptive pill) and MOCP users.

Conclusions

Gender differences exist in the knee joint stiffness properties of young active populations. Females exhibit a lower level of MS and MAS when compared to males. The mechanism explaining this difference is still unknown, but neuromuscular control and muscle volume differences may affect MS and MAS. This study's results may provide some interpretation as to why females incur more knee injuries than their male counterparts. Investigation of optimal training programmes for the augmentation of MS and MAS should be of interest in future.

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Figure

Captions to illustrations

Fig. 1. Myometer was utilized to evaluate c~~C~~ontracted MS~~ measurement technique~~. MS = muscle stiffness

Fig. 2. MAS measurement with free oscillation technique. MAS = musculoarticular stiffness

Fig. 3. Comparison of n~~N~~ormalized peak torque (peak torque/body mass) between males and females (Mean±SD (Standard Deviation)).

* indicates statistically significant difference compared to males.

Fig. 4. Comparison of r~~R~~elaxed MS between males and females (Mean±SD). MS = muscle stiffness

* indicates statistically significant difference compared to males.

Fig. 5. Comparison of c~~C~~ontracted MS between males and females (Mean±SD). MS = muscle stiffness

* indicates statistically significant difference compared to males.

Fig. 6. Comparison of MAS between males and females (Mean±SD). MAS = musculoarticular stiffness

* indicates statistically significant difference compared to males.

Fig. 7. Relationship between MAS of the knee joint and applied load. MAS = musculoarticular stiffness

MAS increased with applied load in both genders. Linear regressions between stiffness and applied load for the male and female populations are significantly different in slope (Males, Y= 36.92X-786.51, r^2 = 0.80; Females, Y= 18.32X+224.49, r^2 = 0.33).

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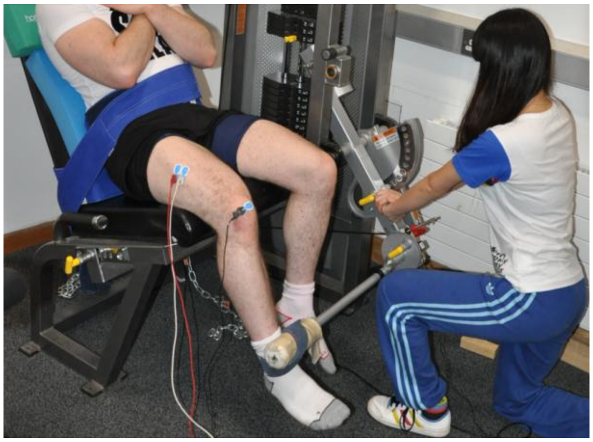
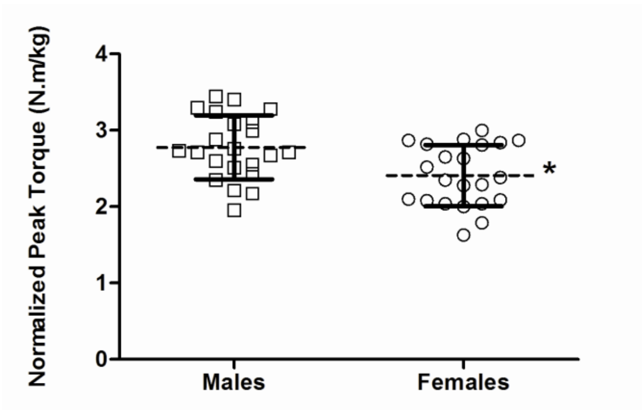
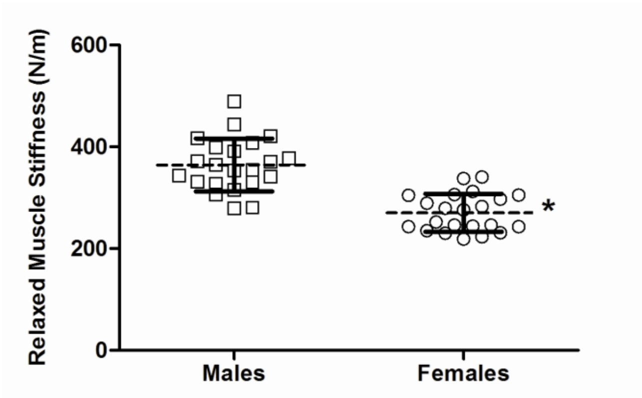
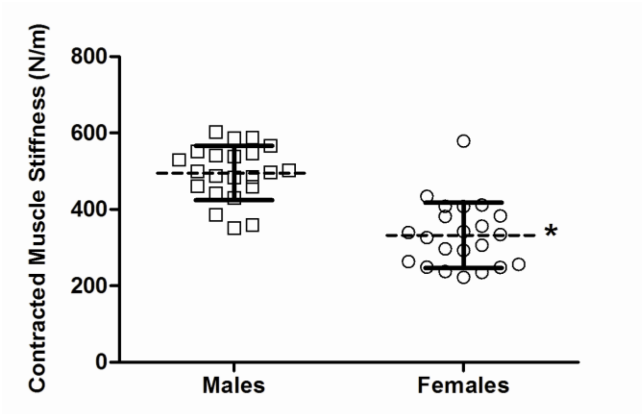




Normalized Peak Torque (N.m/kg)
4
3
2
1
0
*
Males
Females


Relaxed Muscle Stiffness (N/m)
600
400
200
0
Males
Females
*


Contracted Muscle Stiffness (N/m)
800
600
400
200
0
Males
Females
*